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Determination of Thiophosphates in
Industrial and Municipal Wastewaters - Aspon,
Dichlofenthion, Famphur, Fenitrothion,
Fonophos, Phosmet, and Thionazin

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DETERMINATION OF THIOPHOSPHATES IN INDUSTRIAL AND
MUNICIPAL WASTEWATERS - ASPON, DICHLOFENTHION,
FAMPHUR, FENITROTHION, FONOPHOS, PHOSMET, AND
THIONAZIN

by

J.S. Warner, T.M. Engel, and P.J. Mondron,
Battelle Columbus Laboratories
Columbus, Ohio 43201

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Project Officer

Thomas Pressley

Physical and Chemical Methods Branch
Environmental Monitoring and Support Laboratory
Cincinnati, Ohio 45268

ENVIRONMENTAL MONITORING AND SUPPORT LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
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FOREWORD

Environmental measurements are required to determine the quality of ambient waters and the character of waste effluents. The Environmental Monitoring and Support Laboratory - Cincinnati, conducts research to:

- o Develop and evaluate methods to measure the presence and concentration of physical, chemical, and radiological pollutants in water, wastewater, bottom sediments, and solid wastes.
- o Investigate methods for the concentration, recovery and identification of viruses, bacteria, and other microbiological organisms in water; and, to determine the responses to aquatic organisms to water quality.
- o Develop and operate an Agency-wide quality assurance program to assure standardization and quality control of systems for monitoring water and wastewater.
- o Develop and operate a computerized system for instrument automation leading to improved data collection, analysis, and quality control.

This report is one of a series that investigates the analytical behavior of selected pesticides and suggests a suitable test procedure for their measurement in wastewater. The method was modeled after existing EPA methods being specific yet as simplified as possible.

Robert L. Booth, Director
Environmental Monitoring and Support
Laboratory - Cincinnati

ABSTRACT

A method was developed for the determination of seven thiophosphate compounds (aspon, dichlofenthion, famphur, fenitrothion, fonophos, phosmet, and thionazin) in wastewaters. The method development program consisted of a literature review; determination of extraction efficiency for each compound from water into methylene chloride; development of a deactivated Florisil cleanup procedure; and determination of suitable gas chromatographic (GC) analysis conditions. Extraction and cleanup procedures were developed to provide 85 percent or greater recoveries of these compounds from water. A packed column GC-AFD analysis procedure was also developed.

The final method was applied to wastewater from a manufacturer of malathion and to Columbus Publicly Owned Treatment Works (POTW) secondary effluent in order to determine the precision and accuracy of the method. The wastewaters were spiked with the seven compounds at levels of 50 $\mu\text{g/L}$ and 500 $\mu\text{g/L}$, respectively. Recoveries for the seven compounds were in the 85 to 89 percent range at both concentration levels. Method detection limits (MDLs) for the seven compounds in distilled water were in the 1 to 20 $\mu\text{g/L}$ range. MDLs in wastewaters may be higher due to interfering compounds.

This report was submitted in partial fulfillment of Contract No. 68-03-2956 by Battelle Columbus Laboratories under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period from August 1, 1981 to September 30, 1983.

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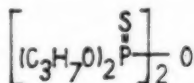
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SECTION 1

INTRODUCTION

The group of compounds designated as "thiophosphates" for method development and validation is comprised of seven thiophosphate compounds: aspon, dichlofenthion, famphur, fenitrothion, fonophos, phosmet, and thionazin. These compounds were not expected to appear simultaneously in wastewaters, but were sufficiently alike in structure and properties to warrant development of one method for all seven pesticides.

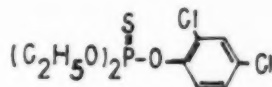
Aspon (I) is a non-systemic insecticide used to control cinch bugs in turf.



I

The CAS registry number for aspon is 3244-90-4 and the IUPAC name is O,O,O',O'-tetrapropyl dithiopyrophosphate. The acute oral LD50 of this compound is 891-1700 mg/kg in rats. Other commonly used synonyms for aspon are "A 42", "ASP 51", "E 8573", and "NPD". Aspon is stable in water and is persistent in soil. No information regarding determination of aspon in any matrix was obtained from a literature search.

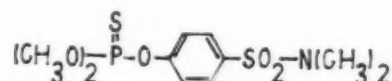
Dichlofenthion (II) is a non-systemic nematocide and soil insecticide.



II

The CAS registry number for dichlofenthion is 97-17-6 and the IUPAC name is 0-2,4-dichlorophenyl 0,0-diethyl phosphorothioate. Its acute oral LD50 in rats is 270 mg/kg. Other common synonyms for dichlofenthion include "VC-13", "ENT 17,470", "ECP", "Nemacide", "VC-13 Nemacide", "V-Cl 13", and "Hexanema". Dichlofenthion is stable to hydrolysis except in extremely alkaline conditions. A literature survey yielded analysis procedure descriptions including the use of benzene and hexane as extraction solvents (1, 2), and the use of Florisil (3) and Sephadex LH-20 (4) for sample cleanup. Gas chromatographic (GC) conditions were described using a flame photometric detector (FPD) (1) and an alkali flame detector (AFD) (2) and packed GC columns including 5% DOW-11, 3% OV-17, and 4% SE-30 (2). In addition, physical properties (5, 6) and mass spectra (7) were reported.

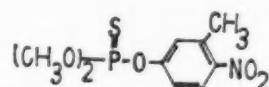
Famphur (III) is an insecticide with a CAS registry number of 52-85-7 and IUPAC name 0,0-dimethyl-O- [p-(dimethylsulfamoyl)phenyl] phosphorothioate.



III

The oral LD50 in rats for famphur is 36-62 mg/kg. Common synonyms for famphur include "ENT 25644", "CL 38023", "AC 38023", "Famophos", "Warbex", "Famfur", "Famphos", and "Varbex". A literature survey provided descriptions of Florisil (3) and acetonitrile/hexane partition (2) cleanup procedures, as well as analysis procedures including thin-layer chromatography (2) and GC-AFD using 4% SE-30 (2) and 2% silicone GE SE-96 (3) columns.

Fenitrothion (II) is a contact insecticide.

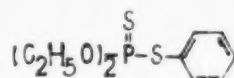


IV

The CAS registry number for fenitrothion is 122-14-5 and the IUPAC name is 0,0-dimethyl 0-4-nitro-m-tolyl phosphorothioate. The LD50 in rats is 500 mg/kg. Fenitrothion is hydrolyzed by alkali. Common synonyms for fenitrothion include "ENT 25715", "OMS 45", and "Sumithion". A

literature search provided large amounts of information on the compound. Several extraction methods were described using various solvents, Tenax (8), and Amberlite XAD (9, 10, 11, 12). Cleanup procedures described included Florisil (13, 14, 3), silica gel (14), thin layer chromatography (TLC) (14), and Sephadex LH-20 (4). Numerous GC analysis methods were described in the above mentioned references using various detectors and columns, as well as an high performance liquid chromatographic (HPLC) method (16). Electron impact mass spectra (EI-MS) (18, 19, 20), toxicity (5, 21), and hydrolysis data (22, 6, 23) were also reported.

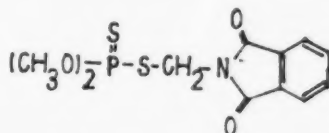
Fonophos (II) is an insecticide suitable for control of soil insects.



V

The CAS registry number for fonophos is 944-22-9 and the IUPAC name is (+)-O-ethyl S-phenyl ethylphosphonodithioate. The oral LD50 in rats for fonophos is 8-18 mg/kg, it is stable under normal storage conditions, and its solubility in water is 13 mg/L. Common synonyms for fonophos include "fonofos", "N 2790", and "Dyfonate". A literature survey yielded descriptions of extractions with methylene chloride (24), benzene (1), and chloroform (25), and cleanup procedures using Bio-Beads SX-3 (25) and Florisil (3). Analysis methods using GC with electron capture (1) and flame photometric (24, 25, 26) detectors and columns such as 4% SE-30/6% SP-2401 (1), 4.6% OV-101/5.5% OV-210 and 3% OV-101 (24), 1.5% OV-17/2% OV-210 (25), and 10% DC-200 (26) were also described. Phosphorescence data (27) and EI-MS data (17) were also reported.

Phosmet (VI) is a non-systemic acaricide and insecticide.

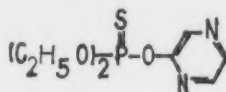


VI

Its CAS registry number is 732-11-6 and its IUPAC name is 0,0-dimethyl S-phthalimidomethyl phosphorodithioate. The oral LD50 in rats of phosmet is 350 mg/kg and it is relatively stable in water except under alkaline conditions. Common synonyms for phosmet include "Imidathion",

"R 1504", "Imidan", "Phthalophos", and "Prolate". A literature survey yielded descriptions of extraction techniques using methylene chloride (28), benzene (29), and Amberlite XAD-2 resin (30). A Florisil cleanup techniques was described (3, 28). Analysis techniques using TLC (31) and GC using alkali flame (28, 30, 32) and flame photometric (29) detectors (FPD) and columns such as DEGS and OV-101 (28), 1.5%/1.95% OV-17/QF-1 (29), 3% OV-17 (30), 2% SF-06 and 10% DC-200 (32) were also described. General information on phosmet (6, 32) as well as EI-MS (18, 20), toxicity (21), and hydrolysis (33) data were also reported.

Thionazin (VII) is a soil insecticide and nematicide of short persistence.



VII

The CAS registry number for thionazin is 297-97-2 and the IUPAC name is O,O-diethyl O-pyrazin-2-yl phosphorothioate. The oral LD50 in rats of thionazin is 12 mg/kg and it is readily hydrolyzed by alkali. Common synonyms for thionazin include "Zinophos", "Nemophos", "Cyanophos", and "EN 18133". A literature review yielded descriptions of cleanup procedures using Florisil (3), and Sephadex LH-20 (4). Analysis procedures were described using GC-FPD with an Apiezon L column (23) and GC-AFD with 2% SF-96 (3) and 4% OV-17 (4) columns. Hydrolysis information (23, 15) and toxicity data (21) were also reported.

These seven compounds are neutral, relatively stable in water under neutral or acidic conditions, easily extracted from water, and contain phosphorous and/or nitrogen. For these reasons, the selected approach to the determination of these compounds in water included extraction from water into methylene chloride using separatory funnel techniques, cleanup using Florisil adsorption chromatography, and analysis using packed column GC-AFD. Standard concentration techniques using Kuderna-Danish (K-D) equipment were employed. The final method is included in Appendix A of this report.

SECTION 2

CONCLUSIONS

EXTRACTION AND CONCENTRATION

The seven thiophosphates can be extracted into methylene chloride from water with greater than 85 percent recovery using standard separatory funnel extraction techniques. Use of Kuderna-Danish (K-D) equipment to perform extract concentrations did not lead to significant recovery losses.

CLEANUP

The thiophosphates elute from six percent deactivated Florisil in 15 percent acetone in ethyl ether or less polar solvents. Recoveries greater than 90 percent are obtained. This use of deactivated Florisil was an effective cleanup procedure for extracts from a Columbus POTW secondary effluent and for extracts from a wastewater from a manufacturer of malathion.

CHROMATOGRAPHY

Two packed GC columns, 3% SP-2250 and 3% SP-2100, were found to be acceptable for the GC-AFD analysis of these compounds. The 3% SP-2250 column gave better peak shape for the thiophosphates and was designated as the primary column. The 3% SP-2100 column was used as the secondary column.

VALIDATION STUDIES

Recoveries of the seven thiophosphates from water at concentrations ranging from 10 to 1000 $\mu\text{g/L}$ were generally greater than 85 percent resulting in linear analytical curves. The method detection limits (MDLs) ranged from 0.6 to 2.2 $\mu\text{g/L}$ for all of the compounds except famphur, which had an MDL of 19 $\mu\text{g/L}$. Recoveries of the thiophosphates from wastewater from a manufacturer of malathion at the 50 $\mu\text{g/L}$ level and from Columbus POTW secondary effluent at the 500 $\mu\text{g/L}$ level were greater than 85 percent.

SECTION 3

EXPERIMENTAL

Studies were performed to determine if techniques such as separatory funnel extraction, Florisil adsorption chromatography cleanup, K-D apparatus concentration, and packed column GC-AFD analysis would be applicable to the determination of the seven thiophosphates in wastewaters. Since recovery data and literature references indicated that these compounds are relatively stable in water, stability studies were not performed.

EXTRACTION AND CONCENTRATION

One liter of reagent water was placed in a two-liter separatory funnel and spiked with 100 µg/L of each of the seven compounds. The pH was adjusted to 7 by addition of 6 N sodium hydroxide or 6 N sulfuric acid. The sample was then extracted three times with 60 mL each of methylene chloride. The combined extracts were dried by passing them through 10 cm of anhydrous granular sodium sulfate and then concentrated to one mL.

CLEANUP

Activated Florisil, 20 grams, was stirred with 100 mL of acetone and 0.4 mL of reagent water for 30 minutes. The slurry was transferred to a chromatographic column, and the solvent was allowed to elute and discarded. The column was then washed with 25 mL of petroleum ether. Petroleum ether, 50 mL, was suspended over the Florisil. The seven compounds, 100 µg of each dissolved in one mL of methylene chloride, were added to the above mentioned petroleum ether. This solvent was eluted from the column and collected (F1). Nine additional 50 mL solvent elutions were collected: six percent ethyl ether in petroleum ether (F2), 15 percent ethyl ether in petroleum ether (F3), 50 percent ethyl ether in petroleum ether (F4), ethyl ether (F5), six percent acetone in ethyl ether (F6), 15 percent acetone in ethyl ether (F7), 50 percent acetone in ethyl ether (F8), acetone (F9), and six percent methanol in acetone (F10). Each fraction was concentrated to one mL.

CHROMATOGRAPHY

Two columns were evaluated for the determination of the seven thiophosphates by GC-AFD: 3% SP-2250 on 100/120 mesh-Supelcoport and 3% SP-2100 on 100/120 mesh-Supelcoport.

VALIDATION STUDIES

The MDLs for the seven compounds were determined by analyzing seven replicate distilled water samples spiked at the 5 $\mu\text{g/L}$ concentration level. The sample extracts were cleaned up using the Florisil cleanup procedure prior to analysis. The amounts recovered were determined by external standard calibration and the MDLs were calculated from these data.

Distilled water was also spiked in duplicate at the 10, 50, 100, 500, and 1000 $\mu\text{g/L}$ concentration levels and recoveries of the seven compounds were determined as described earlier. An analytical curve was generated by plotting the amount spiked into the samples versus the amount recovered from the samples.

A wastewater from a manufacturer of malathion and Columbus POTW secondary effluent were used for method validation studies. Seven aliquots of each wastewater were analyzed to determine levels of interferences. The malathion wastewater was spiked with the seven thiophosphates at the 50 $\mu\text{g/L}$ level, processed and analyzed. The Columbus POTW secondary effluent was spiked at the 500 $\mu\text{g/L}$ level and also processed and analyzed. Seven replicate extractions were performed at each concentration level.

SECTION 4

RESULTS AND DISCUSSION

EXTRACTION AND CONCENTRATION

The seven thiophosphates were extracted from distilled water into methylene chloride with generally greater than 85 percent recovery. Recovery data from duplicate extractions at the 100 and 500 $\mu\text{g/L}$ concentration levels are given in Table 1.

TABLE 1. EXTRACTION OF THIOPHOSPHATES FROM DISTILLED WATER

Compound	Recovery at Given Concentration, % (a)	
	100 $\mu\text{g/L}$	500 $\mu\text{g/L}$
Aspon	90, 93	90, 82
Dichlofenthion	95, 110	96, 85
Famphur	91, 95	110, 110
Fenitrothion	110, 120	80, 69
Fonophos	94, 110	110, 99
Phosmet	85, 110	100, 98
Thionazin	95, 110	120, 110

(a) Results from duplicate analyses.

CLEANUP

The seven thiophosphates eluted in Florisil fractions 3-7 which were obtained by elution with 15 percent ethyl ether in petroleum ether, 50 percent ethyl ether in petroleum ether, ethyl ether, 6 percent acetone in ethyl ether, and 15 percent acetone in ethyl ether, respectively. Recoveries of the compounds were greater than 90 percent. Elution order and recovery data are given in Table 2.

TABLE 2. ELUTION ORDERS AND RECOVERIES OF THIOPHOSPHATES FROM FLORISIL

Compound	Recovery in Specified Fraction, % (a)							Total
	F1	F2	F3	F4	F5	F6	F7	
Aspon			94	2				96
Dichlofenthion			92					92
Famphur						6	103	109
Fenitrothion				51	55			106
Fonophos			84	6				90
Phosmet						37	69	106
Thionazine				2	93			95

(a) Results of single determination with 100 µg of each compound.

(b) Elution solvents were 50 mL each of the following:

- F1 = 2% methylene chloride in petroleum ether
- F2 = 6% ethyl ether in petroleum ether
- F3 = 15% ethyl ether in petroleum ether
- F4 = 50% ethyl ether in petroleum ether
- F5 = 100% ethyl ether
- F6 = 6% acetone in ethyl ether
- F7 = 15% acetone in ethyl ether

CHROMATOGRAPHY

Both an 3% SP-2250 column and an 3% SP-2100 column were satisfactory for the GC determination of the seven thiophosphates. The 3% SP-2250 column, however, gave better peak shape and was chosen as the primary column. The following conditions were used for the primary column:

Column: 2m x 0.2mm I.D. 3% SP-2250 on 100/120 mesh Supelcoport
 Detector: Alkali flame detector (AFD), 16 bead volts
 Injector Temperature: 250°C
 Detector Temperature: 300°C
 Oven Temperature: 80°C isothermal for 4 min; programmed from 80°C to 300°C at 8°C/min; 300°C isothermal for 4 min.
 Carrier Gas: Helium at 30 mL/minute

A chromatogram obtained under these conditions is shown in Figure 1.

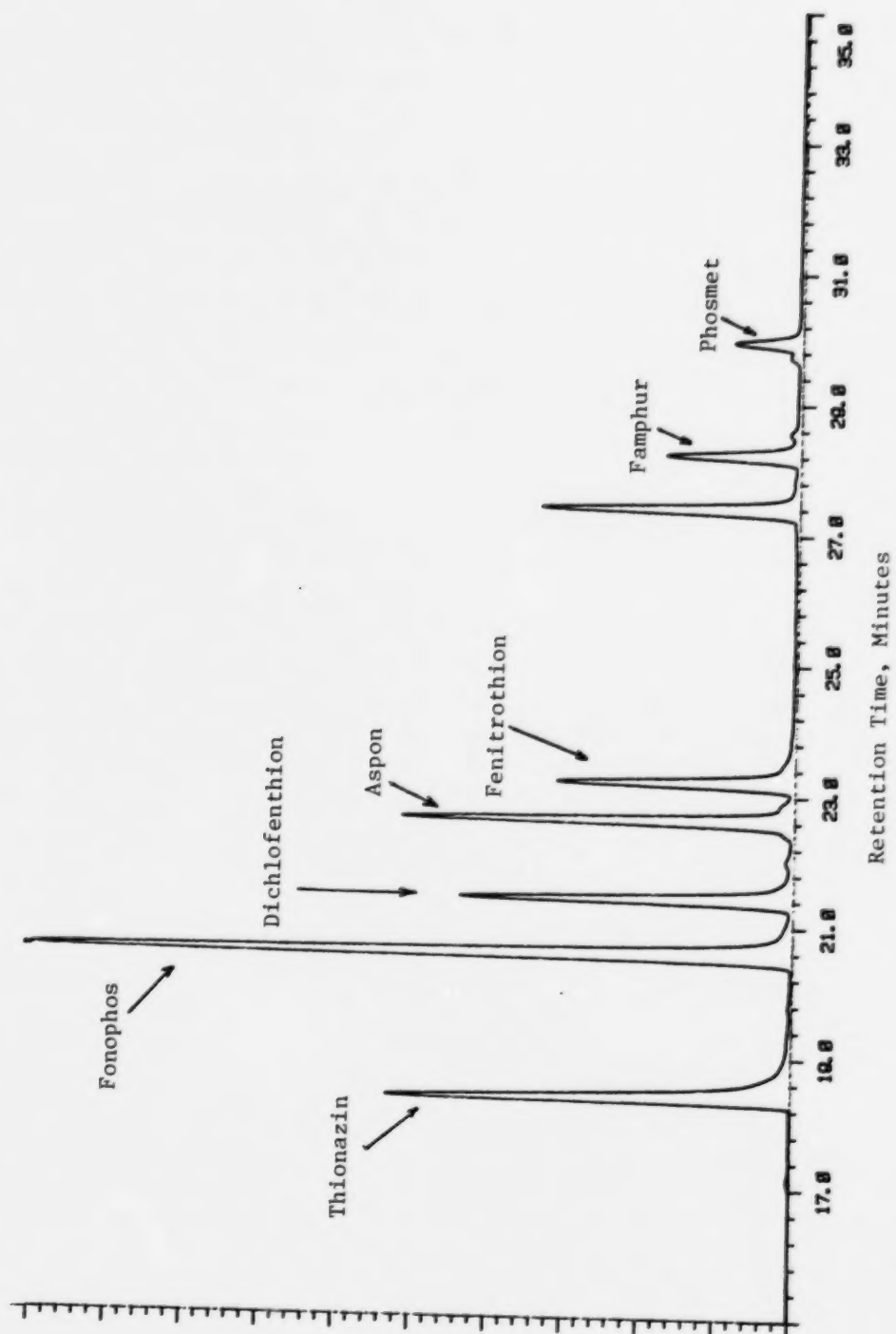


Figure 1. GC-AFD Chromatogram of 100 ng Each of the Seven Thiophosphates (Column 1)

The following conditions were used for the alternate column:

Column: 2m x 0.2 mm I.D. 3% SP-2100 on 100/120 mesh Supelcoport
Detector: Alkali flame detector, 16 bead volts
Injector Temperature: 250°C
Detector Temperature: 250°C
Oven Temperature: 80°C isothermal for 10 min; programmed from 80°C to 300°C at 8°C/min; 300°C isothermal for 4 min.
Gas Carrier: Helium at 30 mL/minute

A chromatogram obtained under these conditions is shown in Figure 2.

VALIDATION STUDIES

MDLs for the thiophosphates generally ranged from 0.6 to 2.2 µg/L. The MDL for famphur was much higher (19 µg/L). This high MDL does not reflect any difficulties in detection of famphur, two ng of which is easily detected using the GC-AFD conditions described earlier. Rather it was a result of a high standard deviation for the data due to an interfering peak of unknown origin. Recoveries and MDL data are given in Table 3.

TABLE 3. DATA FROM MDL DETERMINATION FOR THE THIOPHOSPHATES

Compound	Amount Spiked, µg/L	Amount Recovered, µg/L(a)	MDL, µg/L
Aspon	5.0	4.4 ± 0.2	0.6
Dichlofenthion	5.0	4.3 ± 0.2	0.7
Famphur	5.0	12 ± 6	19
Fenitrothion	5.0	6.0 ± 0.7	2.2
Fonophos	5.0	4.2 ± 0.2	0.7
Phosmet	5.0	4.2 ± 0.4	1.3
Thionazin	5.0	5.1 ± 0.4	1.2

(a) Average of seven replicate distilled water analyses.

Recoveries of the thiophosphates obtained using the analytical curve studies were greater than 85 percent except at the 10 µg/mL concentration level for aspon, fonophos, and phosmet. The resultant analytical curves were linear in the concentration range of 10 to 1000 µg/L. Recovery data are given in Table 4. The analytical curves generated from the recovery data are shown in Figures 3-9.

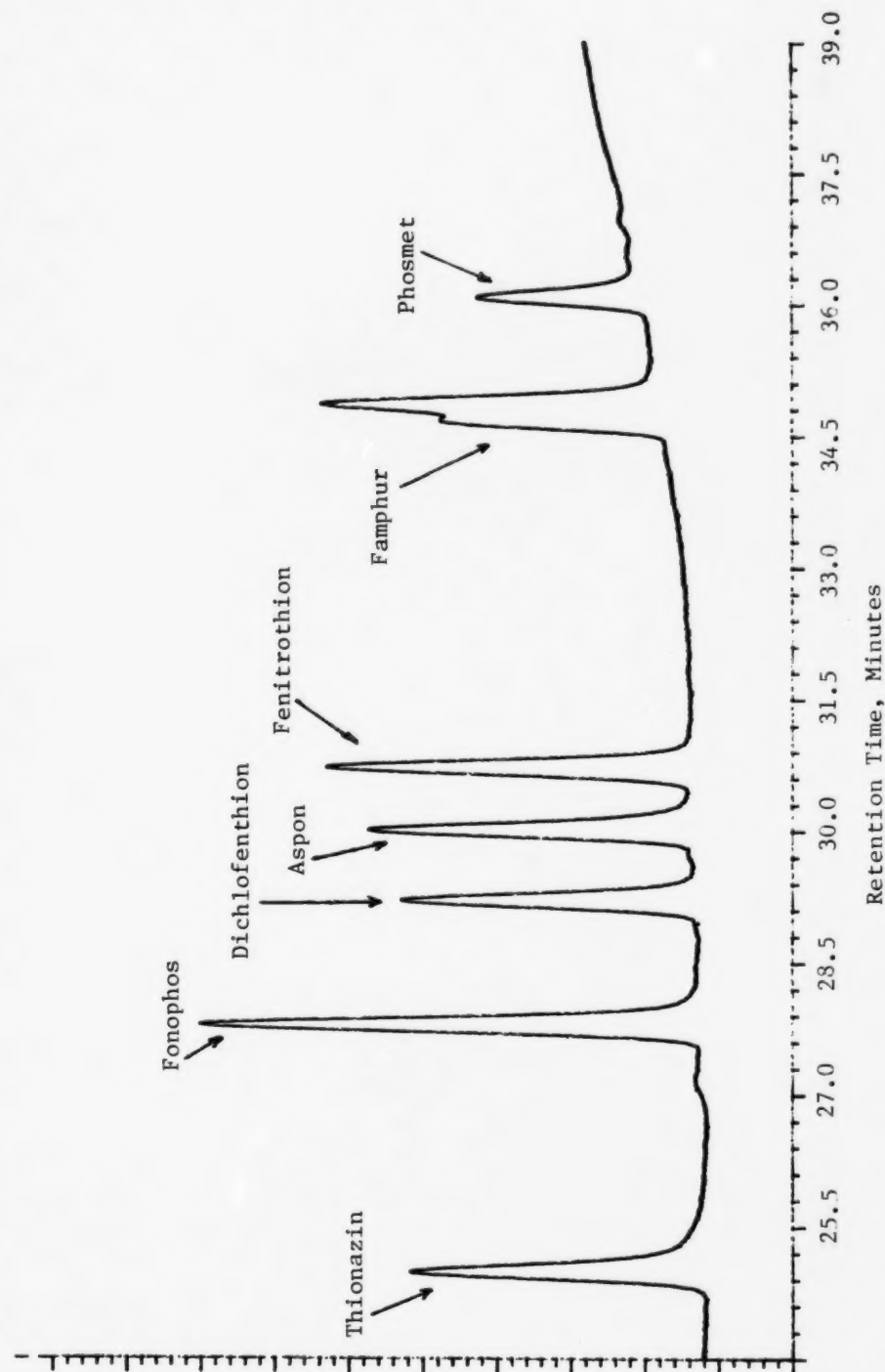


Figure 2. GC-FID Chromatogram of 100 ng Each of Seven Thiophosphates (Column 2)

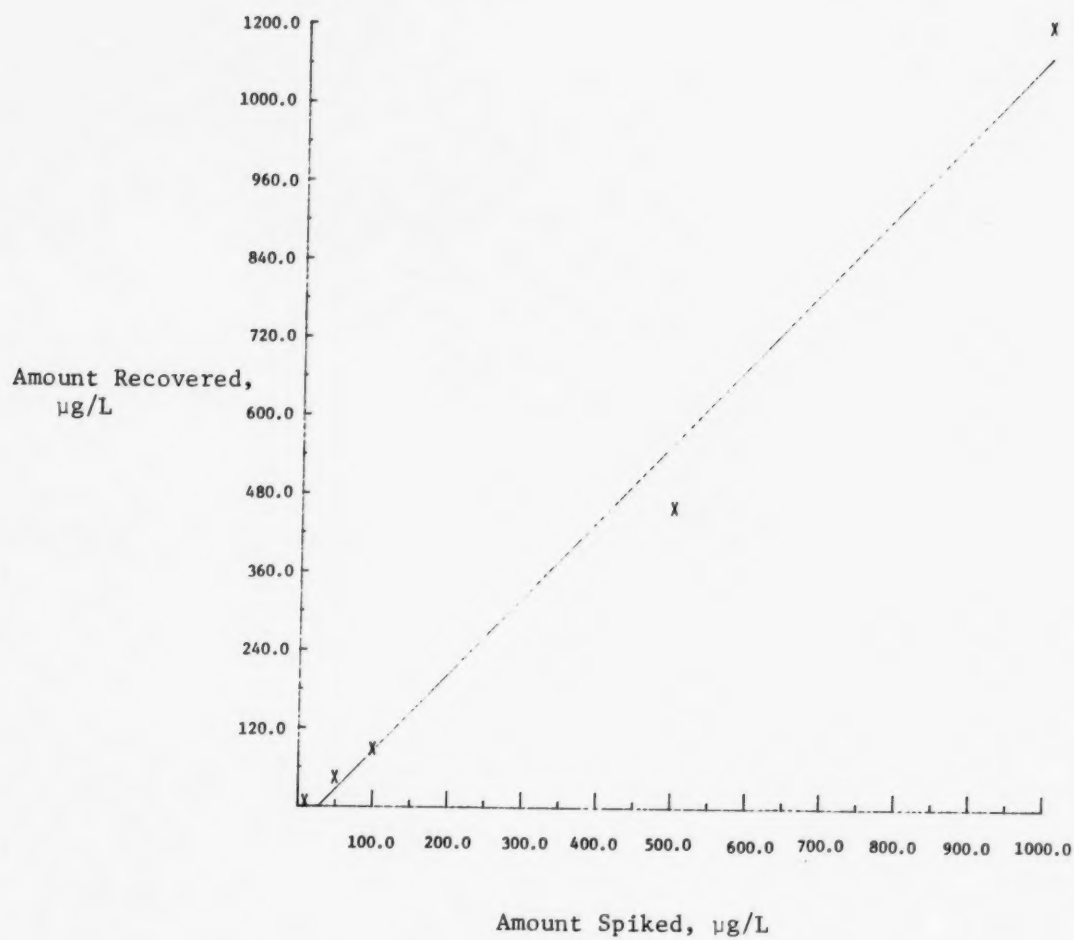


Figure 3. Analytical Curve for Aspon.

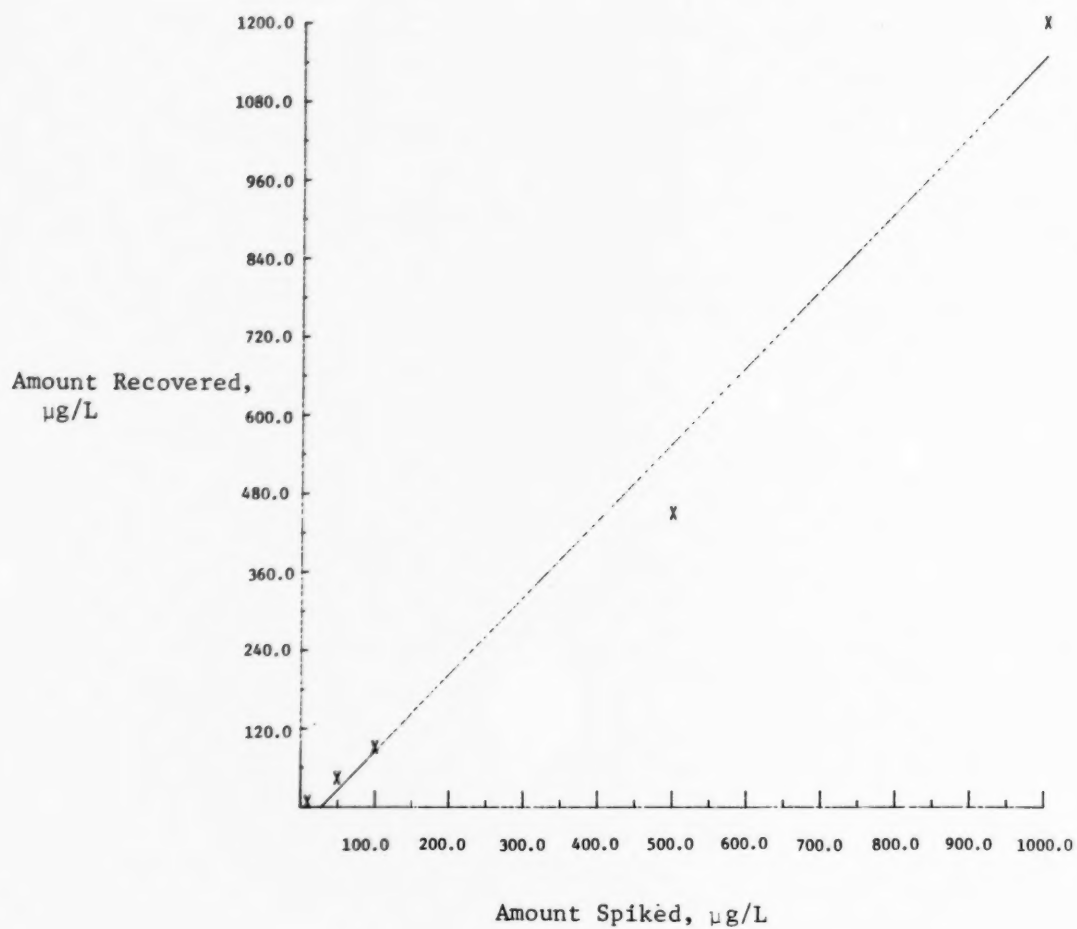


Figure 4. Analytical Curve for Dichlofenthion.

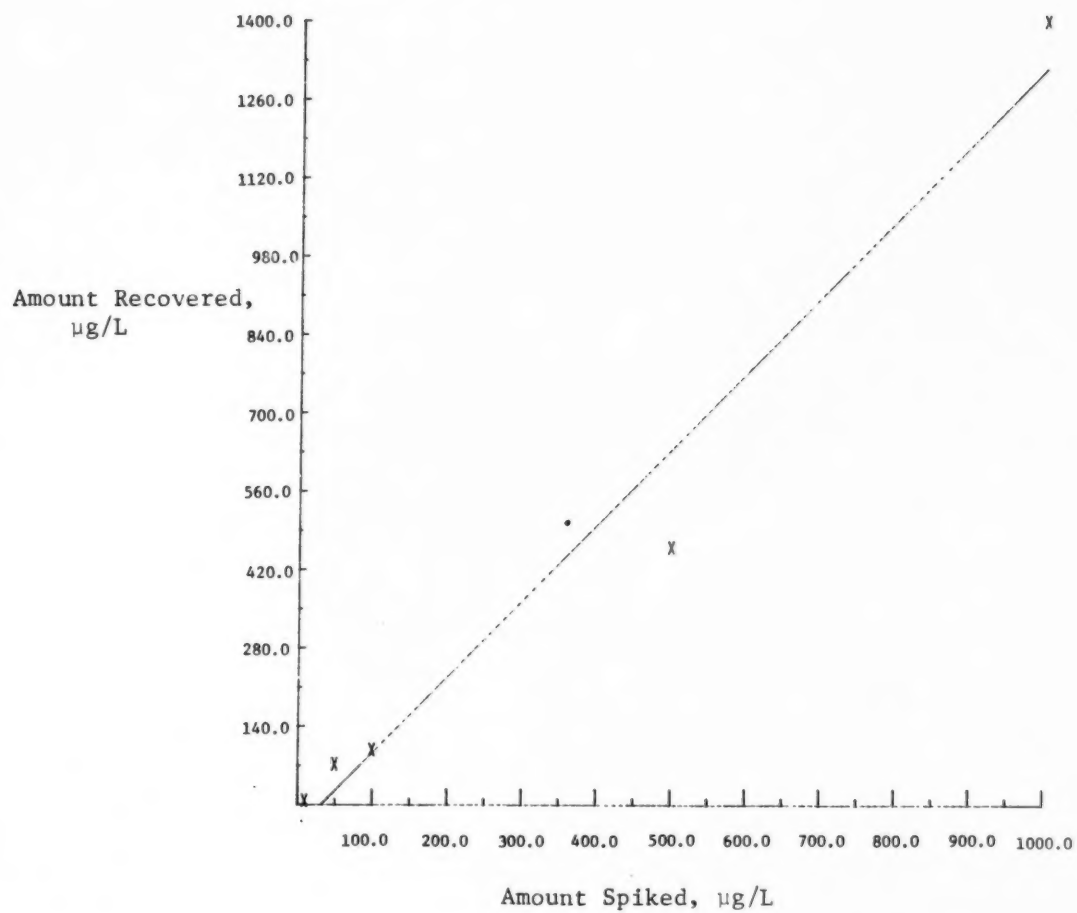


Figure 5. Analytical Curve for Famphur.

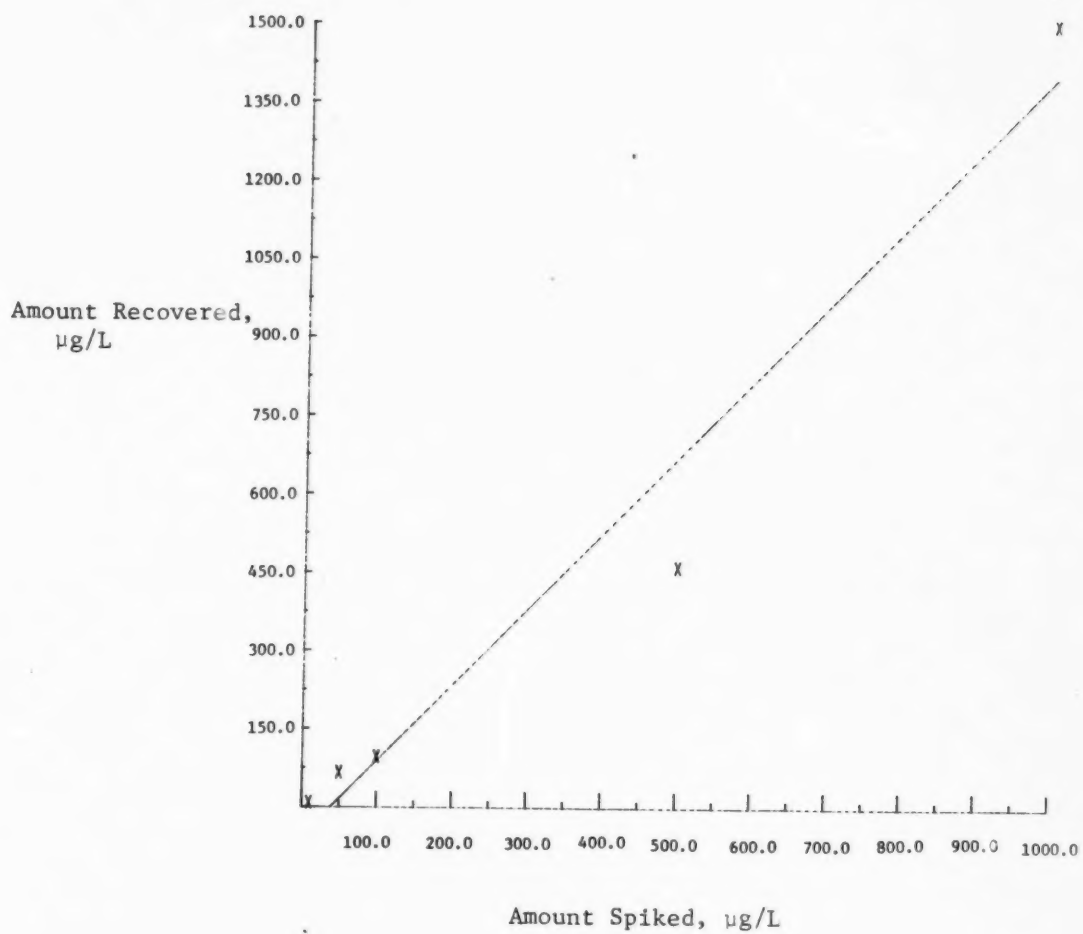


Figure 6. Analytical Curve for Fenitrothion.

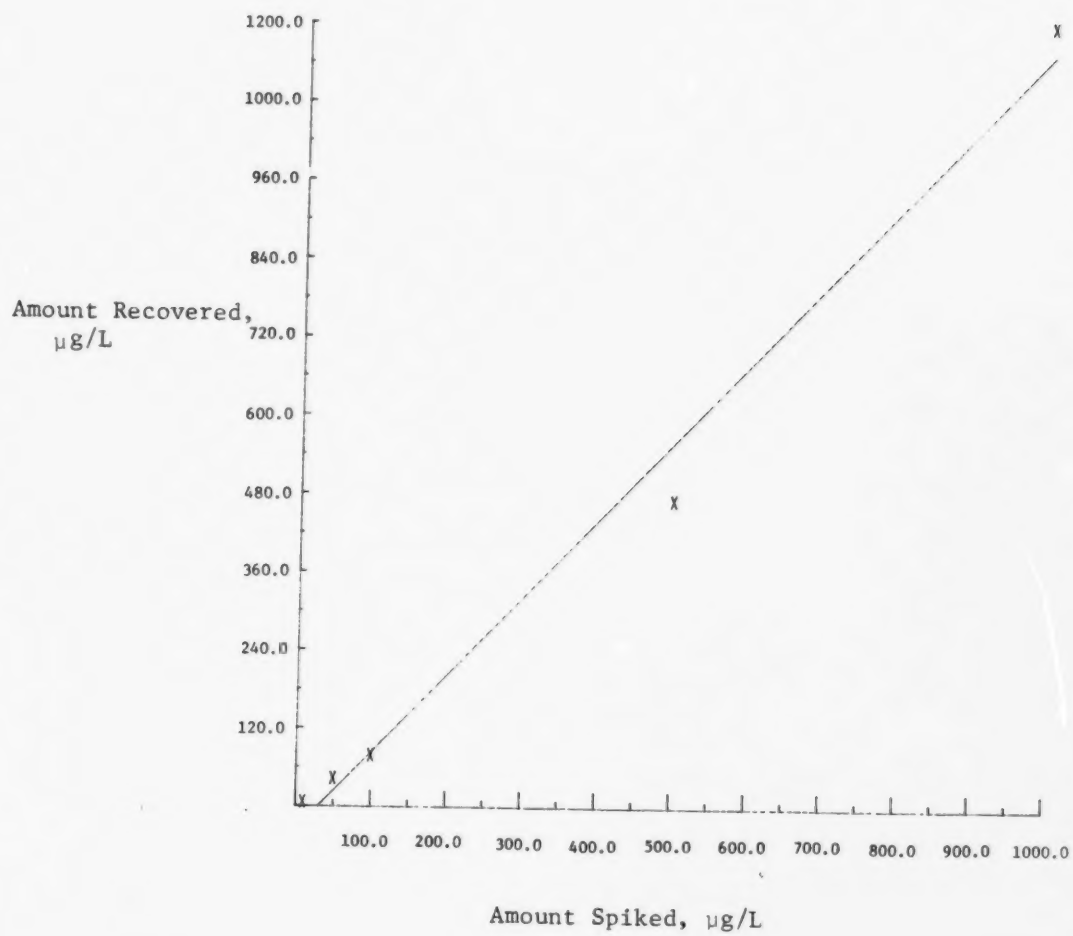


Figure 7. Analytical Curve for Fonophos.

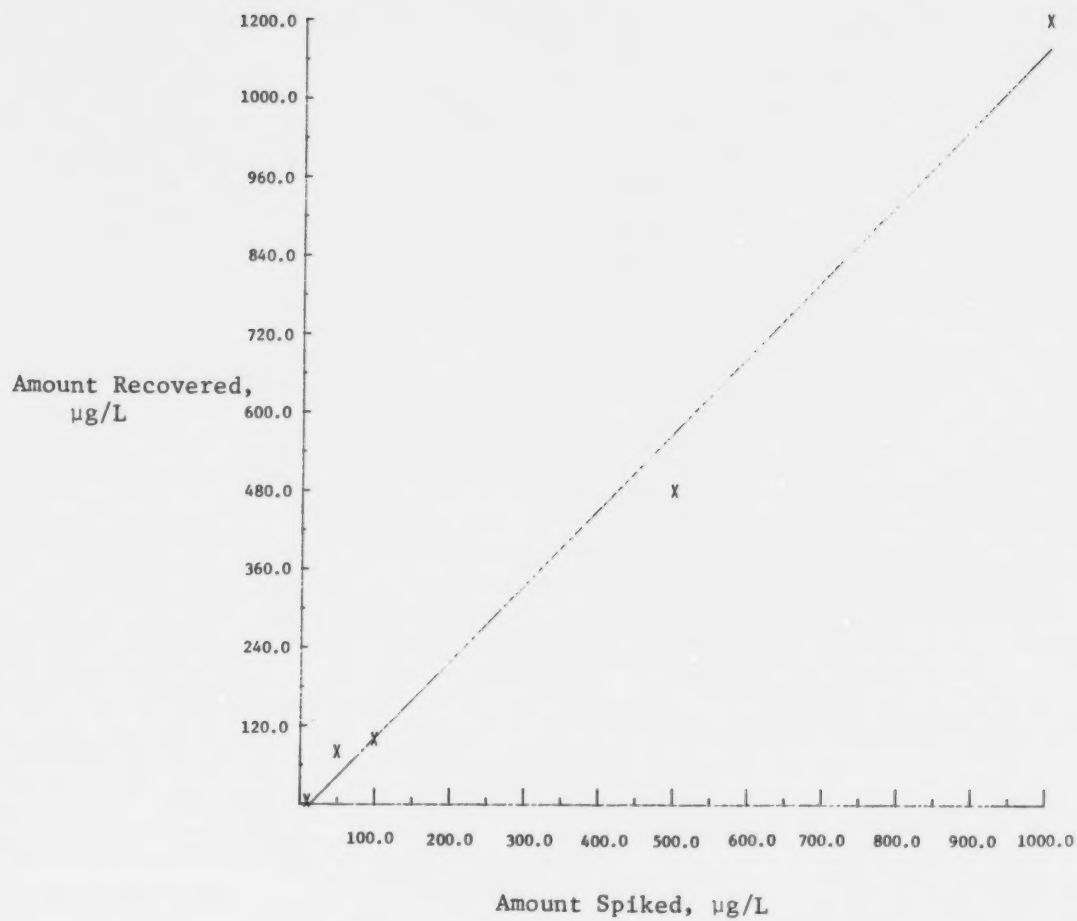


Figure 8. Analytical Curve for Phosmet.

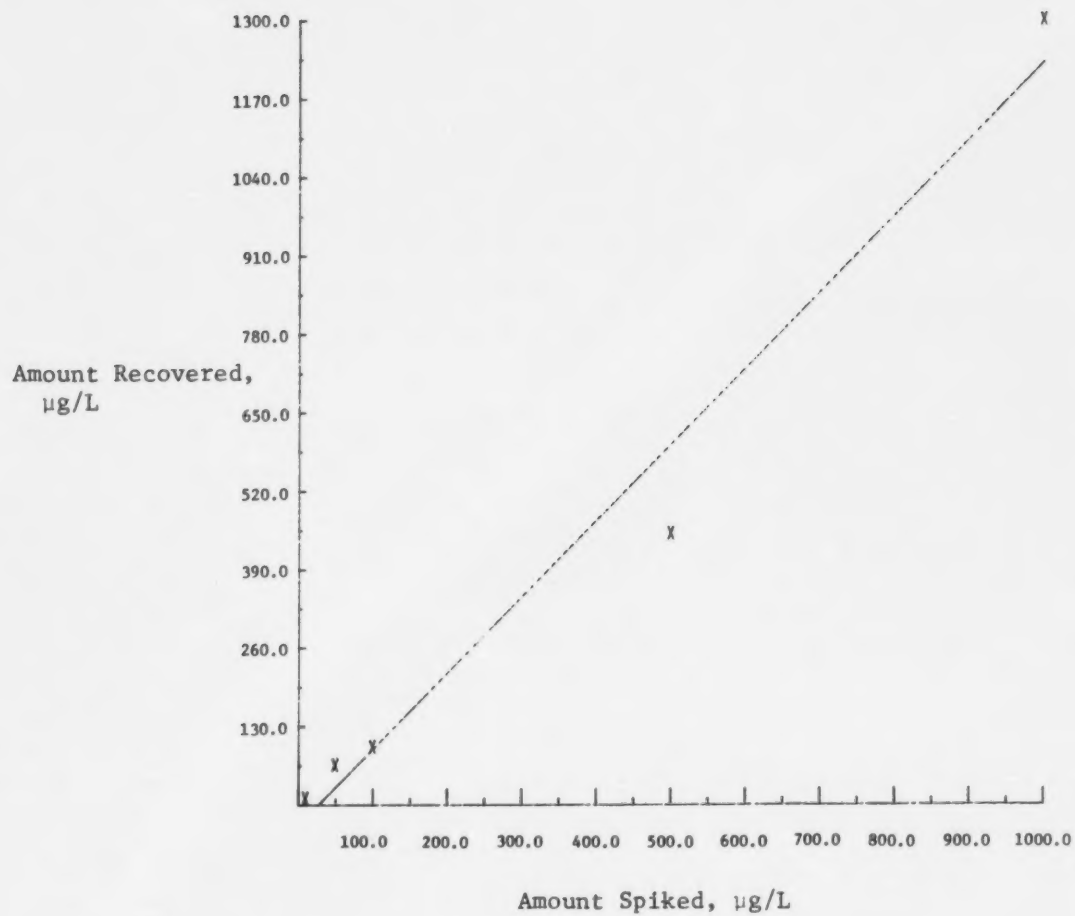


Figure 9. Analytical Curve for Thionazin.

TABLE 4. DATA FROM ANALYTICAL CURVE DETERMINATION FOR THE THIOPHOSPHATES

Compound	Amount Recovered at Given Spike Level, $\mu\text{g/L(a)}$				
	10	50	100	500	1000
Aspon	8.0(12)	45(44)	89(16)	460(7.4)	1200(11)
Dichlofenthion	8.5(17)	45(60)	92(14)	450(11)	1200(6.0)
Famphur	8.3(29)	73(14)	99(34)	460(11)	1400(3.5)
Fenitrothion	9.1(23)	67(6.7)	97(28)	450(12)	1500(4.7)
Fonophos	6.8(30)	43(5.4)	79(11)	470(5.1)	1200(5.0)
Phosmet	5.2(3.1)	81(3.1)	100(5.2)	480(0.3)	1200(3.4)
Thionazin	12(34)	67(13)	97(31)	450(16)	1300(6.1)

(a) Average of duplicate distilled water analyses; percent relative range given in parenthesis.

Recoveries of the thiophosphates from wastewater at the 50- and 500- $\mu\text{g/L}$ levels were in the range of 82 to 89 percent. Recovery data from the analyses of thiophosphates in Columbus POTW secondary effluent and wastewater from a manufacturer of malathion are given in Table 5. Chromatograms of Columbus POTW secondary effluent, unspiked and spiked at the 500 $\mu\text{g/L}$ level, and of wastewater from a manufacturer of malathion, unspiked and spiked at the 50 $\mu\text{g/L}$ level, are shown in Figures 10 and 11, respectively. No interferences were observed in the unspiked wastewaters.

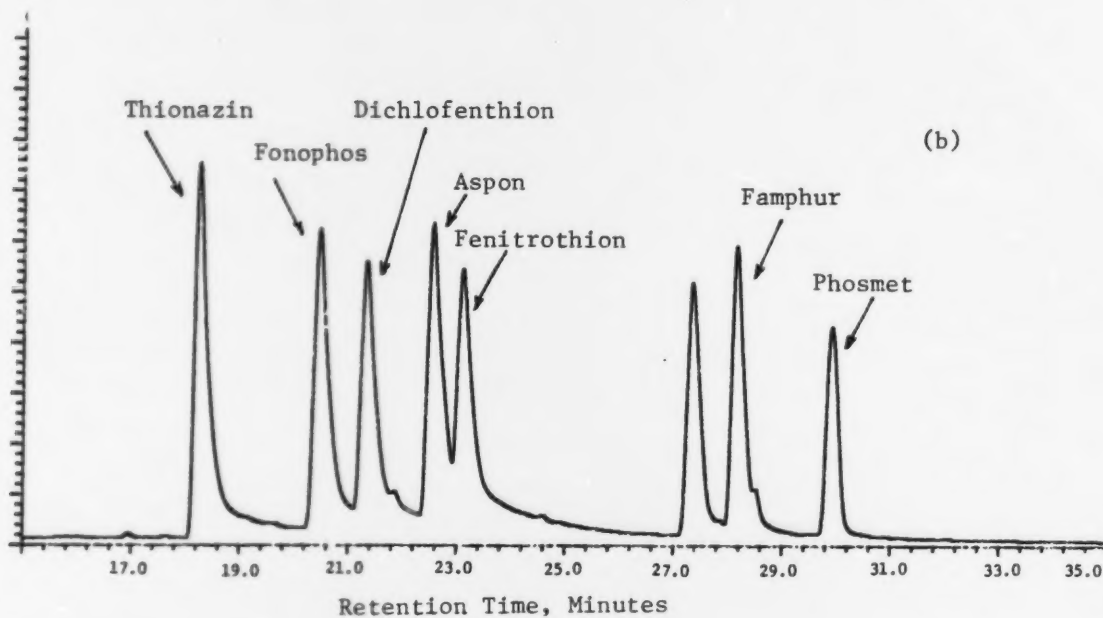
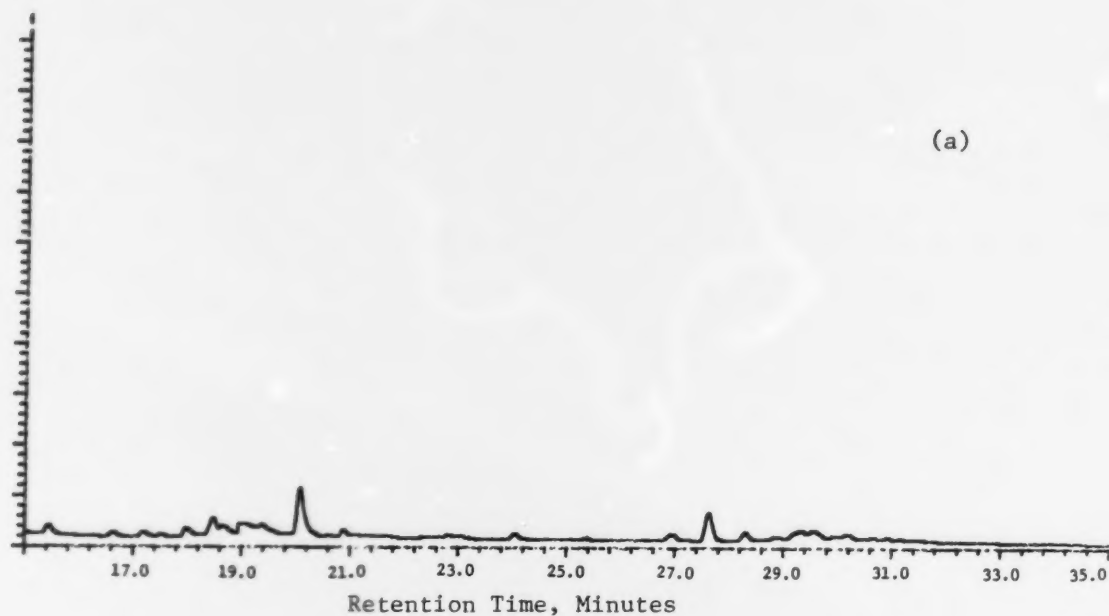


Figure 10. GC-AFD Chromatograms of Columbus POTW Secondary Effluent
(a) Unspiked and (b) Spiked at the 500 $\mu\text{g/L}$ Level with
the Seven Thiophosphates.

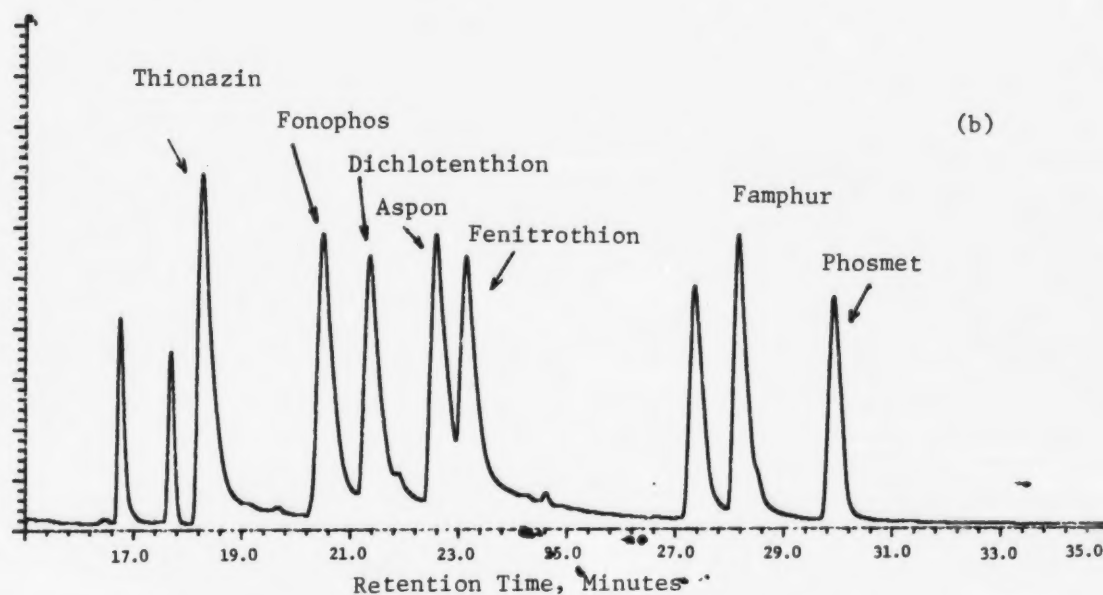
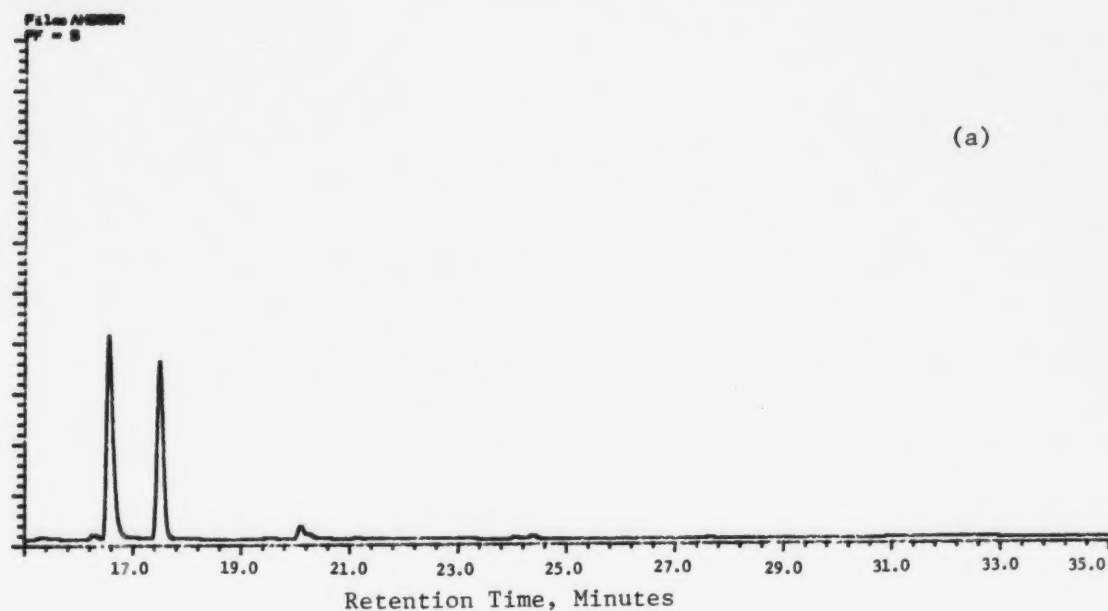


Figure 11. GC-AFD Chromatograms of Wastewater from a Manufacturer of Malathion (a) Unspiked and (b) Spiked at the 50 $\mu\text{g/L}$ Level with the Seven Thiophosphates.

TABLE 5. RECOVERIES OF THIOPHOSPHATES FROM WASTEWATER

Compound	Wastewater(a)	Spike	Average	Relative Standard Deviation, %
		Level µg/L	Recovery, % µg/L(b)	
Aspon	1	50	83	6.1
	2	500	87	3.2
Dichlofenthion	1	50	83	5.8
	2	500	84	2.9
Famphur	1	50	86	5.4
	2	500	86	3.2
Fenitrothion	1	50	82	5.6
	2	500	83	2.8
Fonophos	1	50	84	6.2
	2	500	86	3.2
Phosmet	1	50	85	4.3
	2	500	87	3.8
Thionazin	1	50	84	5.9
	2	500	89	3.7

(a) 1 = Wastewater from a manufacturer of malathion.
 2 = Columbus POTW secondary effluent.

(b) Average of seven replicate analyses.

REFERENCES

1. Coburn, J.A., and A.S.Y. Chan. Confirmation of Pesticide Residue Identity: Part IX Organophosphorus Pesticides. Environmental Letters, 10(3):225-236, 1975.
2. Clear, M.H., F.R. Fowler, S.R.B. Solly, and A.R. Ritchie. Detection of Organophosphorus and Carbamate Pesticides in Adipose Tissue by Thin-layer and Gas-Liquid Chromatography. New Zealand Journal of Science, 20:221-224, 1977.
3. Beckman, H. and D. Garber. Recovery of 65 Organophosphorus Pesticides from Florisil with a New Solvent Elution System. Journal of the AOAC, 52(2):286-293, 1969.
4. Pflugmacher, J. and W. Ebing. Cleanup of Organophosphorus Insecticide Residues in Vegetable Extracts by Gel Chromatography on Sephadex LH-20. Journal of Chromatography, 93:457-463, 1974.
5. Freed, V.H., R. Hague, D. Schmedding, and R. Kohnert. Physicochemical Properties of some Organophosphates in Relation to Their Chronic Toxicity. Environmental Health Perspectives, 13:77-81, 1976.
6. Freed, V.H., D. Schmedding, R. Kohnert, and R. Hague. Physical Chemical Properties of Several Organophosphates: Some Implication in Environmental and Biological Behavior. Pesticide Biochemistry and Physiology, 10:203-211, 1979.
7. Busch, K.L., M.M. Bursey, J.R. Hass, and G.W. Sovocool. Comparison of Five Ionization Methods for Producing Mass Spectra of Typical Organophosphorus Pesticides. Applied Spectroscopy, 32(4):388-399, 1978.
8. Leoni, V., G. Puccetti, and A. Grella. Preliminary Results on the Use of Tenax for the Extraction of Pesticides and Polynuclear Aromatic Hydrocarbons from Surface and Drinking Waters for Analytical Purposes. Journal of Chromatography, 106:119-124, 1975.
9. Mallet, V.N., J.M. Francoeur, and G. Volpe. Evaluation of Amberlite XAD-4 for the Recovery of Fenitrothion from Water. Journal of Chromatography, 172:388-393, 1979.

10. Berkane, K., G.E. Caissie, and V.N. Mallet. The Use of Amberlite XAD-2 Resin for the Quantitative Recovery of Fenitrothion from Water - A Preservation Technique. Journal of Chromatography, 139:386-390, 1977.
11. Mallet, V.N., G.L. Brun, R.N. MacDonald, and K. Berkane. A Comparative Study of the Use of XAD-2 Resin and the Conventional Serial Solvent Extraction Procedure for the Analysis of Fenitrothion and Some Derivatives in Water-Preservation Techniques, Journal of Chromatography, 160:81-88, 1978.
12. Volpe, G., and V.N. Mallet. Recovery of Fenitrothion from Water Using Amberlite Resin XAD-7. Journal of Chromatography, 117:385-389, 1979.
13. Dale, W.E., and J.W. Miles. Partition Chromatographic Separation of Pesticide Residues from Fats. Journal of the AOAC, 59(1):165-168, 1976.
14. Takimoto, V. and J. Miyamoto. Residue Analysis of Sumithion. Residue Rev., 60:83-101, 1976.
15. Faust, S.D. and H.M. Gomaa. Chemical Hydrolysis of Some Organic Phosphorus and Carbamate Pesticides in Aquatic Environments. Environmental Letters, 3(3):171-201, 1972.
16. Lawrence, J.F., C. Renault, and R.W. Frei. Fluorogenic Labeling of Organophosphate Pesticides with Dansyl Chloride Application to Residue Analysis by High-Pressure Liquid Chromatography and Thin-Layer Chromatography. Journal of Chromatography, 121:343-351, 1976.
17. Mestres, R., Ch. Chevallier, Cl. Espinoza, and R. Cornet. Application of Coupled Gas-Chromatography-Mass Spectrometry in Methods for the Study and Determination of Pesticide Residues and Organic Micropollutants in Environmental and Food Materials, Ann. Falsif. Expert. Chim., 77(51):178-188, 1970.
18. Damico, J.N.. The Mass Spectra of Some Organophosphorus Pesticide Compounds. Journal of the AOAC, 49(5):1027-1045, 1966.
19. Wolfenbarger, D.A., and T.N. Shaver. An Infrared spectrophotometric Technique for Determining Residues of Several Organophosphorus Insecticides from Cotton Foliage. Journal of Economic Entomology, 66(2):332-336, 1973.

20. Stan, J.H., B. Abraham, J. Jung, M. Kellert, and K. Steinland. Detection of Organophosphorus Insecticides by Gas Chromatography/Mass Spectrometry. Fresenius Z. Anal. Chem., 287:271-285, 1977.
21. Gaines, T.B. Acute Toxicity of Pesticides. Toxicology and Applied Pharmacology, 14:515-534, 1969.
22. Maguire, R.J., and E.J. Hale. Fenitrothion Sprayed on a Pond: Kinetics of Its Distribution and Transformation in Water and Sediment. J. Agric. Food Chem., 28:372-378, 1980.
23. Ruzicka, J.Y., J. Thomson, and B.B. Wheals. The Gas Chromatographic Determination of Organophosphorus Pesticides Part II. A Comparative Study of Hydrolysis Rates. Journal of Chromatography, 31:37-47, 1967.
24. Bourne, S. Preservation of Organophosphorous Pesticides in Water Samples. J. Environ. Sci. Health, B13(2):75-86, 1978.
25. Ault, J.A., C.M. Schofield, L.D. Johnson, and R.H. Waltz. Automated Gel Permeation Chromatographic Preparation of Vegetables, Fruits, and Crops for Organophosphate Residue Determination Utilizing Flame Photometric Detection. J. Agric. Food. Chem., 27(4):825-828, 1979.
26. Barney, J.E., W.Y. Ja, and M.S. O'Connor. Dyfonate. In: Analytical methods for Pesticides and Plant Growth Regulators (Volume VII), Gunter Zweig, ed. Academic Press, New York and London, 1973. pp. 269-277.
27. Aaron, J.J., E.M. Daleel, and J.D. Winefordner. Comparative Study of Low-Temperature and Room-Temperature Phosphorescence Characteristics of Several Pesticides. J. Agric. Food Chem., 27(b):1233-1237, 1979.
28. Luke, M.A., J.E. Froberg, and H.T. Masumoto. Extraction and Cleanup of Organochlorine, Organophosphate, Organonitrogen, and Hydrocarbon Pesticides in Produce for Determination by Gas-Liquid Chromatography. Journal of the AOAC, 58(5):1020-1042, 1974.
29. Ripley, B.D., R.J. Wilkinson, and A.S.Y. Chan. Multiresidue Analysis of Fourteen Organophosphorus Pesticides in Natural Waters. Journal of the AOAC, 57(5):1033-1042, 1974.

30. LeBel, G.L., D.T. Williams, G. Griffith, and F.M. Benoit. Isolation and Concentration of Organophosphorus Pesticides from Drinking Water at the ng/L Level using Macroreticular Resin. J. Assoc. Off. Anal. Chem., 62(2):241:249, 1979.
31. Schutzmann, R.L., and W.F. Barthel. Indoxyl Acetate Spray Reagent for Fluorogenic Detection of Cholinesterase Inhibitors in Environmental Samples. Journal of the AOAC, 52(1):151-156, 1969.
32. Barney, J.E., W.Y. Ja. and D.L. Shelman. Imidan. In: Analytical Methods for Pesticides and Plant Growth Regulators (Volume VI), Gunterzweig, ed. Academic Press, New York, San Francisco, and London, 1977. pp. 408-413.
33. Freed, V.H., C.T. Chiou, and D.W. Schmedding. Degradation of Selected Organophosphate Pesticide in Water and Soil. J. Agric. Food Chem., 27(4):706-708, 1979.

THIOPHOSPHATE PESTICIDES

METHOD 622.1

1. Scope and Application

- 1.1 This method covers the determination of certain thiophosphate pesticides. The following parameters can be determined by this method:

<u>Parameter</u>	<u>CAS No.</u>
Aspon	3244-90-4
Dichlofenthion	97-17-6
Famphur	52-85-7
Fenitrothion	122-14-5
Fonophos	944-22-9
Phosmet	732-11-6
Thionazin	297-97-2

- 1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 CFR 136.1. Any modification of this method beyond those expressly permitted, shall be considered a major modification subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.
- 1.3 The method detection limit (MDL, defined in Section 15) for each parameter is listed in Table 2. The MDL for a specific wastewater

wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

- 1.4 The sample extraction and concentration steps in this method are essentially the same as in certain other 600 series methods. Thus, a single sample may be extracted to measure the compounds included in the scope of the methods. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, in order to apply appropriate cleanup procedures.
- 1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.
- 1.6 When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Section 14 provides gas chromatograph/mass spectrometer (GC-MS) criteria appropriate for the qualitative confirmation of compound identifications.

2. Summary of Method

- 2.1 A measured volume of sample, approximately 1 liter, is solvent extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and concentrated to 1.0 mL.

Gas chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by alkali flame detector gas chromatography (GC-AFD).¹

2.2 This method provides a Florisil column cleanup procedure to aid in the elimination of interferences which may be encountered.

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned.² Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15-30 min. Do not heat volumetric ware. Some thermally stable materials such as PCBs may not be eliminated by this treatment. Thorough rinsing with acetone and pesticide quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. The cleanup procedure in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 2.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified³⁻⁵ for the information of the analyst.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle - Amber borosilicate or flint glass, 1-liter or 1-quart volume, fitted with screw caps lined with Teflon. Foil may be substituted for Teflon if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional) - Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only.)

5.2.1 Separatory funnel - 2000-mL, with Teflon stopcock.

5.2.2 Drying column - Chromatographic column 400 mm long x 10 mm ID with coarse frit.

5.2.3 Chromatographic column - 400 mm long x 19 mm ID with 250 mL reservoir at the top and Teflon stopcock (Kontes K-420290 or equivalent).

- 5.2.4 Concentrator tube, Kuderna-Danish - 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. A ground glass stopper is used to prevent evaporation of extracts.
- 5.2.5 Evaporative flask, Kuderna-Danish - 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.
- 5.2.6 Snyder column, Kuderna-Danish - three-ball macro (Kontes K-503000-0121 or equivalent).
- 5.2.7 Snyder column, Kuderna-Danish - two-ball micro (Kontes K-569001-0219 or equivalent).
- 5.2.8 Vials - Amber glass, 10 to 15 mL capacity with Teflon lined screw-cap.
- 5.2.9 Erlenmeyer flask - 250-mL
- 5.2.10 Graduated cylinder - 1000-mL
- 5.3 Boiling chips - approximately 10/40 mesh carborundum. Heat at 400°C for 4 hours or Soxhlet extract with methylene chloride.
- 5.4 Water bath - Heated, capable of temperature control ($\pm 2^{\circ}\text{C}$). The bath should be used in a hood.
- 5.5 Balance - Analytical, capable of accurately weighing to the nearest 0.0001 g.
- 5.6 Gas chromatograph - Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.

- 5.6.1 Column 1 - 180 cm long x 2 mm ID glass, packed with 3% SP2250 on Supelcoport (100/120 mesh) or equivalent. This column was used to develop the method performance statements in Section 15. Alternate columns may be used in accordance with the provisions described in Section 12.1.
- 5.6.2 Column 2 - 180 cm long x 2 mm ID glass, packed with 3% SP-2100 on Supelcoport (100/120 mesh) or equivalent.
- 5.6.3 Detector - Alkali-flame detector (AFD), sometimes referred to as a nitrogen-phosphorous detector (NPD) or a thermionic specific detector (TSD). This detector has proven effective in the analysis of wastewaters for the compounds listed in the scope and was used to develop the method performance statements in Section 15.

6. Reagents

- 6.1 Reagent water - Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.
- 6.2 Methylene chloride, methanol, petroleum ether, anhydrous ethyl ether, and acetone distilled-in-glass quality or equivalent. Ethyl ether must be free of peroxides as indicated by EM Quant Test Strips (available from Scientific Products Co., Catalog No. P 1126-8 and other suppliers). Procedures recommended for removal of peroxides are provided with the test strips.
- 6.3 Sodium sulfate (ACS) Granular, anhydrous; heated in a muffle furnace at 400°C overnight.

6.4 Florisil - PR grade (60/100 mesh). Purchase activated at 1250 F and store in brown glass bottle. To prepare for use, place 150 g in a wide-mouth jar and heat overnight at 160-170°C. Seal tightly with Teflon or aluminum foil-lined screw cap and cool to room temperature.

6.5 6N Sodium hydroxide

6.6 6N Sulfuric acid

6.7 Stock standard solutions (1.00 $\mu\text{g}/\mu\text{L}$) - Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

6.7.1 Prepare stock standard solutions by accurately weighing about 0.0100 grams of pure material. Dissolve the material in distilled-in-glass quality ethyl ether and dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

6.7.2 Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store at 4°C and protect from light. Frequently check standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

6.7.3 Stock standard solutions must be replaced after six months or sooner if comparison with check standards indicates a problem.

7. Calibration

7.1 Establish gas chromatographic operating parameters equivalent to those indicated in Table 2. The gas chromatographic system can be calibrated using the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure:

7.2.1 For each compound of interest, prepare calibration standards at a minimum of three concentration levels by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with ethyl ether. One of the external standards should be at a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.

7.2.2 Using injections of 1 to 5 μ L of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each compound. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), can be calculated for each compound at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working

range, the average calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than +10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that compound.

7.3 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Due to these limitations, no internal standard applicable to all samples can be suggested.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each compound of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with ethyl ether. One of the standards should be at a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.

7.3.2 Using injections of 1 to 5 μ L of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

$$RF = (A_S C_{IS}) / (A_{IS} C_S)$$

where:

A_S = Response for the compound to be measured.

A_{IS} = Response for the internal standard.

C_{IS} = Concentration of the internal standard in μ g/L.

C_S = Concentration of the compound to be measured in μ g/L.

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_S/A_{IS} against RF.

7.3.3 The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate

elution patterns and the absence of interferences from the reagents.

8. Quality Control

8.1 Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a

quality control check sample concentrate in methanol 1000 times more concentrated than the selected concentrations.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using the appropriate data from Table 3, determine the recovery and single operator precision expected for the method, and compare these results to the values measured in Section 8.2.3. If the data are not comparable, the analyst must review potential problem areas and repeat the test.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance as follows:

$$\text{Upper Control Limit (UCL)} = R + 3s$$

$$\text{Lower Control Limit (LCL)} = R - 3s$$

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts⁶ that are useful in observing trends in performance.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s . Alternately, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.⁶

8.4 The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular compound does not fall within the control limits for method performance, the results reported for that compound in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst should demonstrate through the analysis of a 1-liter aliquot of reagent water that all

glassware and reagents interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. Samples Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices⁷ should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.

- 9.3 Adjust the pH of the sample to 6 to 8 with 6N sodium hydroxide or 6N sulfuric acid immediately after sampling.

10. Sample Extraction

- 10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-liter separatory funnel. Check the pH of the sample with wide range pH paper and adjust to 6 to 8 with 6 N sodium hydroxide or 6 N sulfuric acid.
- 10.2 Add 60 mL of methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 min with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 min. If the emulsion interface between layers is more than one third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.
- 10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

- 10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.
- 10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer. Once the flask rinse has passed through the drying column, rinse the column with 30 to 40 mL of methylene chloride.
- 10.6 Add 1 or 2 clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 min. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min.
- 10.7 Remove the macro-Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methylene chloride. Add 1 or 2 clean boiling chips and attach a two-ball micro-Snyder column to the concentrator tube. Prewet the

micro-Snyder column with methylene chloride and concentrate the solvent extract as before. When an apparent volume of 0.5 mL is reached, or the solution stops boiling, remove the K-D apparatus and allow it to drain and cool for 10 minutes.

10.8 Remove the micro-Snyder column and adjust the volume of the extract to 1.0 mL with methylene chloride. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extract is to be stored longer than two days, transfer the extract to a screw capped vial with a Teflon-lined cap. If the sample extract requires no further cleanup, proceed with the gas chromatographic analysis in Section 12. If the sample requires cleanup, proceed to Section 11.

10.9 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Cleanup and Separation

11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various clean waters and industrial effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than that reported in Table 3.

11.2 The following Florisil column cleanup procedure has been demonstrated to be applicable to the seven thiophosphate pesticides listed in Table 1.

- 11.2.1 Add 20 g of Florisil to 100 mL of ethyl ether and 400 μ L of reagent water in a 250-mL Erlenmeyer flask. Shake vigorously for 15 minutes. Transfer the slurry to a chromatographic column (Florisil may be retained with a plug of glass wool). Allow the solvent to elute from the column until the Florisil is almost exposed to the air. Wash the column with 25 mL of petroleum ether. Use a column flow of 2 to 2.5 mL/min throughout the wash and elution profiles. Add an additional 50 mL of petroleum ether to the head of the column.
- 11.2.2 Quantitatively add the sample extract from Section 10.8 to the head of the column. Allow the solvent to elute from the column until the Florisil is almost exposed to the air. Elute the column with 50 mL of 6% ethyl ether in petroleum ether. Discard this fraction.
- 11.2.3 Elute the column with 50 mL of 15% ethyl ether in petroleum ether (Fraction 1) and collect eluate in a K-D apparatus. Repeat process with 50 mL of 50% ethyl ether in petroleum ether (Fraction 2), 50 mL of 100% ethyl ether (Fraction 3), 50 mL 6% acetone in ethyl ether (Fraction 4), and 100 mL 15% acetone in ethyl ether (Fraction 5), collecting each fraction in a separate K-D apparatus. The elution patterns for the thiophosphates are shown in Table 1. Concentrate each fraction to 1 mL as described in Sections 10.6 and 10.7. Proceed with gas chromatographic analysis.
- 11.2.4 The above-mentioned fractions can be combined before concentration at the discretion of the analyst.

12. Gas Chromatography

- 12.1 Table 2 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. Examples of the separations achieved by Column 1 and Column 2 are shown in Figures 1 and 2. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.
- 12.2 Calibrate the gas chromatographic system daily as described in Section 7.
- 12.3 If the internal standard approach is being used, the analyst must not add the internal standard to the sample extracts until immediately before injection into the instrument. Mix thoroughly.
- 12.4 Inject 1 to 5 μL of the sample extract using the solvent flush technique⁸. Record the volume injected to the nearest 0.05 μL , and the resulting peak sizes in area or peak height units. An automated system that consistently injects a constant volume of extract may also be used.
- 12.5 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of

the analyst should weigh heavily in the interpretation of chromatograms.

12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. Calculations

13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_t)}{(V_i)(V_s)}$$

where:

A = Amount of material injected, in nanograms.

V_i = Volume of extract injected in μL .

V_t = Volume of total extract in μL .

V_s = Volume of water extracted in mL.

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

$$\text{Concentration, } \mu\text{g/L} = \frac{(A_s)(I_s)}{(A_{is})(\text{RF})(V_o)}$$

where:

A_s = Response for the compound to be measured.

A_{IS} = Response for the internal standard.

I_s = Amount of internal standard added to each
extract in μg .

V_0 = Volume of water extracted, in liters.

13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected compounds must be labeled as suspect.

14. GC-MS Confirmation

14.1 It is recommended that GC-MS techniques be judiciously employed to support qualitative identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the compound. The instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak but not to exceed 7 seconds per scan utilizing a 70-V (nominal) electron energy in the electron impact ionization mode. A GC to MS interface constructed of all-glass or glass-lined materials is recommended. When using a fused silica capillary column, the column outlet should be threaded through the interface to within a few mm of the entrance to the source ionization chamber. A computer system should be interfaced to the mass spectrometer that allows the continuous acquisition and

storage on machine readable media of all mass spectra obtained throughout the duration of the chromatographic program.

14.2 Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC-MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved.¹⁰

14.3 At the beginning of each day that confirmatory analyses are to be performed, the GC-MS system must be checked to see that all DFTPP performance criteria are achieved.⁹

14.4 To confirm an identification of a compound, the background corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. It is recommended that at least 25 nanograms of material be injected into the GC-MS. The criteria below must be met for qualitative confirmation.

14.4.1 The molecular ion and all other ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to plus or minus 10%. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20-40%.

14.4.2 The retention time of the compound in the sample must be within 6 seconds of the same compound in the standard solution.

14.4.3 Compounds that have very similar mass spectra can be explicitly identified by GC-MS only on the basis of retention time data.

14.5 Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.

14.6 Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternate packed or capillary GC columns or additional cleanup (Section 11).

15. Method Performance

15.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.¹¹ The MDL concentrations listed in Table 2 were obtained using reagent water.¹ Similar results were achieved using representative wastewaters.

15.2 This method has been tested for linearity of recovery from spiked reagent water and has been demonstrated to be applicable over the concentration range from 10 x MDL to 1000 x MDL.

15.3 In a single laboratory, Battelle Columbus Laboratories, using spiked wastewater samples, the average recoveries presented in Table 3 were obtained after Florisil cleanup. Seven replicates of each of two different wastewaters were spiked and analyzed. The standard deviation of the percent recovery is also included in Table 3.¹

REFERENCES

1. "Development of Methods for Pesticides in Wastewaters," Report for EPA Contract 68-03-2956 (In preparation).
2. ASTM Annual Book of Standards, Part 31, D3694, "Standard Practice for Preparation of Sample Containers and for Preservation," American Society for Testing and Materials, Philadelphia, PA, p. 679, 1980.
3. "Carcinogens - Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, August, 1977.
4. "OSHA Safety and Health Standards, General Industry," (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206 (Revised, January 1976).
5. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.
6. "Handbook for Analytical Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, U. S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory - Cincinnati, Ohio 45268, March 1979.
7. ASTM Annual Book of Standards, Part 31, D3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA, p. 76, 1980.
8. Burke, J. A., "Gas Chromatography for Pesticide Residue Analysis; Some Practical Aspects," Journal of the Association of Official Analytical Chemists, 48, 1037 (1965).
9. Eichelberger, J. W., Harris, L. E., and Budde, W. L., "Reference Compound to Calibrate Ion Abundance Measurement in Gas Chromatography - Mass Spectrometry," Analytical Chemistry, 47, 995 (1975).
10. McNair, H. M. and Bonelli, E. J., "Basic Chromatography", Consolidated Printing, Berkeley, California, p. 52 (1969).
11. Glaser, J. A. et al, "Trace Analysis for Wastewaters", Environmental Science and Technology, 15, 1426 (1981).

TABLE 1. ELUTION ORDERS AND RECOVERIES OF THIOPHOSPHATES FROM FLORISIL

Compound	Recovery in Specified Fraction, % (a)						
	F1	F2	F3	F4	F5	F6	F7
Aspon			94	2			
Dichlofenthion			92				
Famphur						6	103
Fenitrothion				51	55		
Fonophos			84	6			
Phosmet						37	69
Thionazine				2	93		

(a) Results of single determination with 100 µg of each compound.

(b) Elution solvents were 50 mL each of the following:

- F1 = 2% methylene chloride in petroleum ether
- F2 = 6% ethyl ether in petroleum ether
- F3 = 15% ethyl ether in petroleum ether
- F4 = 50% ethyl ether in petroleum ether
- F5 = 100% ethyl ether
- F6 = 6% acetone in ethyl ether
- F7 = 15% acetone in ethyl ether

TABLE 2. CHROMATOGRAPHIC CONDITIONS AND
ESTIMATED METHOD DETECTION LIMITS

Parameter	Retention Time (Min.)		MDL ($\mu\text{g/L}$)
	Column 1	Column 2	
Thionazin	18.3	25.0	1
Fonophos	20.5	27.8	0.7
Dichlofenthion	21.4	29.4	0.7
Aspon	22.6	30.2	0.6
Fenitrothion	23.1	30.8	2
Famphur	28.1	34.8	19
Phosmet	30.0	36.2	1

Column 1 conditions: Supelcoport (100/120 mesh) coated with 3% SP-2250 packed in a 1.8 m long x 2 mm ID glass column with helium carrier gas at a flow rate of 30 mL/min. Column temperature is programmed from 80°C to 300°C at 8°C/min. with a 4 minute hold at each extreme, injector temperature is 250°C and detector is 300°C. Alkali flame detector at bead voltage of 16 volts.

Column 2 conditions: Supelcoport (100/120 mesh) coated with 3% SP-2100 packed in a 1.8 m long x 2 mm ID glass columns with helium carrier gas at a flow rate of 30 mL/min. Column temperature is programmed from 80°C to 300°C at 8°C/min. with a 4 minute hold at each extreme, injector temperature is 250°C and detector is 300°C.

TABLE 3. SINGLE LABORATORY ACCURACY AND PRECISION(a)

Parameter	Sample Type(b)	Background $\mu\text{g/L}$ (c)	Spike $\mu\text{g/L}$	Mean Recovery %	% Relative Standard Deviation	Number of Replicates
Aspon	1	ND	50	83	7	7
	2	ND	500	87	3	7
Dichlofenthion	1	ND	50	83	7	7
	2	ND	500	84	4	7
Famphur	1	ND	50	86	6	7
	2	ND	500	86	4	7
Fenitrothion	1	ND	50	82	7	7
	2	ND	500	83	4	7
Fonophos	1	ND	50	84	7	7
	2	ND	500	86	4	7
Phosmet	1	ND	50	85	5	7
	2	ND	500	87	5	7
Thionizin	1	ND	50	84	7	7
	2	ND	500	89	5	7

(a) Column 1 conditions were used.

(b) 1 = Low level relevant industrial effluent
2 = Municipal sewage influent.

(c) ND = Not detected.

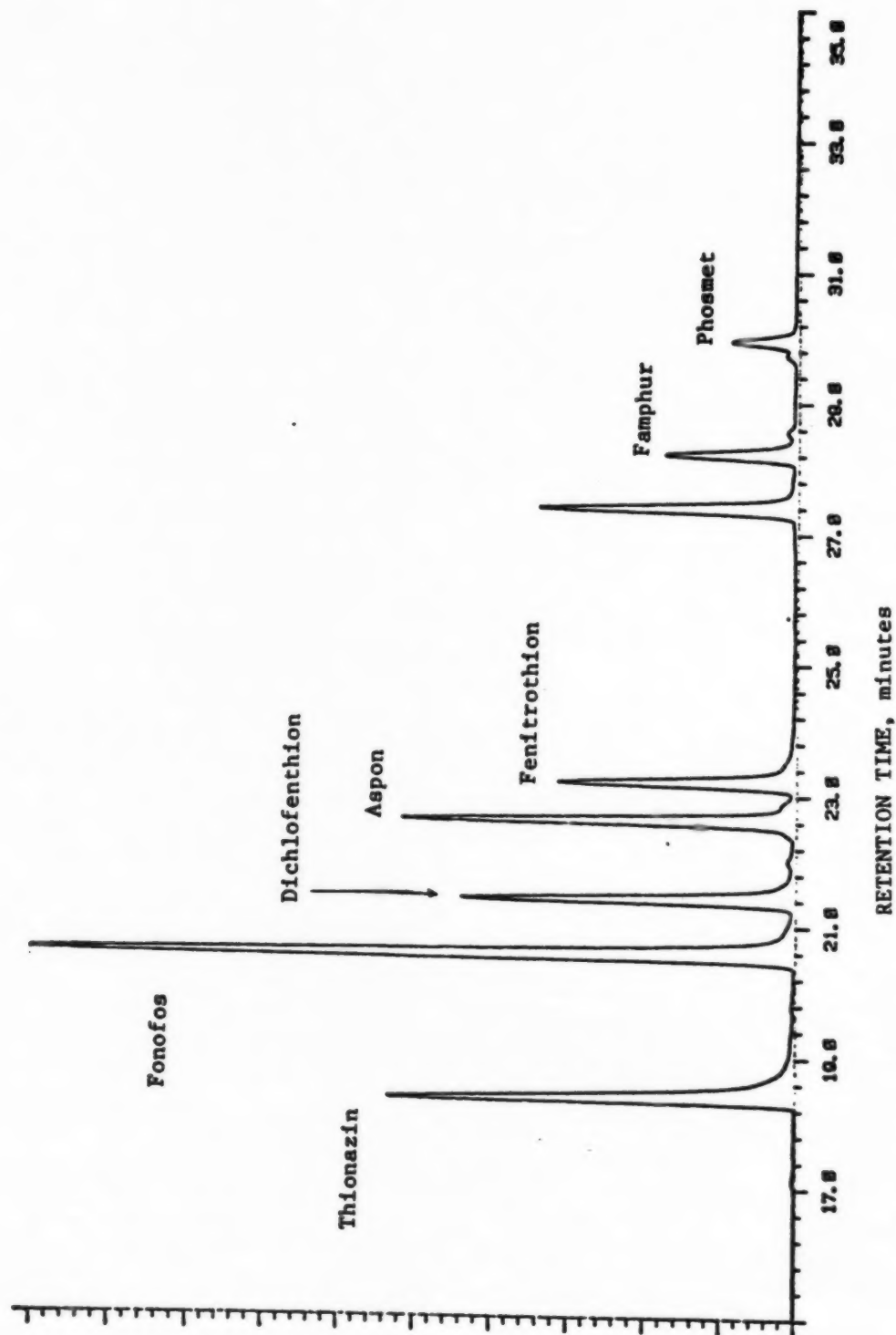


FIGURE 1. GC-AFD CHROMATOGRAM OF 100 ng EACH OF SEVEN THIOPHOSPHATES (COLUMN 1).

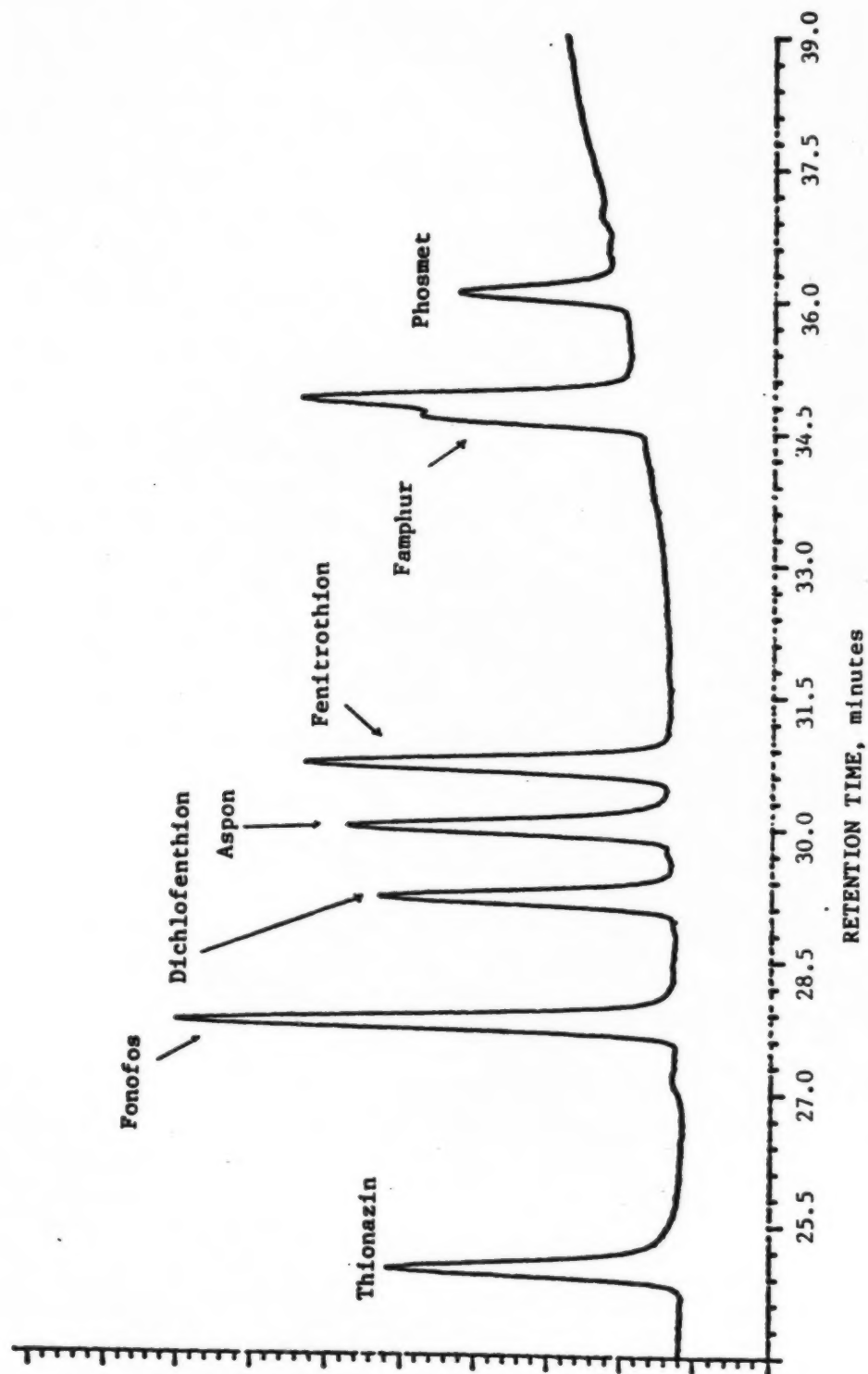


FIGURE 2. GC-FID CHROMATOGRAM OF 100 ng EACH OF SEVEN THIOPHOSPHATES (COLUMN 2).